Communication to the Editor

Are we analysing knockdown in the right way? How independence of the knockdown-recovery process from mortality may affect measures for behavioural effects in pesticide bioassays

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Abstract: In pesticide bioassays, especially those with neurotoxic agents, effects on animals are typically grouped into classes according to behaviour, such as normal and affected behaviour, which may range from unstable walking behaviour, to unable to move, to mortality. Generally, recovery is observed in all these effect classes, except the last. Mortality, however, disturbs the analysis of the recovery processes because it decreases the number of animals that otherwise could have shown a reversible effect. We consider that this interaction between mortality and other, reversible, effects is a conceptual problem, and give arguments in favour of analysing changes in behaviour and mortality as two independent, simultaneously occurring neurotoxic syndromes. As an illustration, two data sets are analysed in both ways and these show that marked differences may exist between conclusions reached by the two viewpoints. The consequences thereof are discussed in relation to toxico-kinetic explanations for neurotoxicant effects on behaviour and mortality.

Keywords: knockdown; neurotoxicity; endocrine disruption; toxicokinetics; pesticide bioassay; data analysis; pyrethroids

1 INTRODUCTION

Neurotoxic pesticides change the functioning of arthropods in several ways. The responses are related to the functions of the parts of the neural system that are affected, and involve different gradations of exaggerated or inhibited behaviour,[1] and exaggerated or inhibited neurosecretion.[2] Eventually, the effects may cause the death of the animal.

Many pesticide bioassays have focused on the immobilisation of animals following pesticide poisoning. The reason is that behavioural effects are considered indicative of the toxic action of pesticides. If immobilisation is very rapid, it is generally referred to as 'knockdown'. Knockdown has been studied most thoroughly for pyrethroids and DDT, where it is particularly important. The general approach to knockdown and other effects on behaviour is to group the behaviour of individuals into separate, well-defined classes. Typically, a minimum of three effect classes is recognised: healthy animals showing no effects; living animals showing a particular change in behaviour, and dead animals. In some cases no distinction is made between affected and dead animals.[3–5] In other cases the animals showing affected behaviour are split into smaller sub-groups on the basis of the intensity of the observed effects.[6,7] A total of four different effect classes was recognised by Toth and Sparks[8] in beetle larvae treated with DDT or pyrethroids: hyperactivity, during which animals showed increased activity and restlessness; ataxia, which was marked by uncoordinated or stumbling movements; prostration, during which animals curled up without being able to right themselves, and a stage which involved convulsions and tremors.

In general, each animal is assigned to its proper class. As a consequence, less knockdown is observed when a toxicant causes high mortality, because animals can only be knocked down or dead (Fig 1). Due to this way of working, the effect curves for knockdown and recovery are affected by mortality and do not exclusively reflect the knockdown-recovery process. We regard this as a handicap for studying knockdown, and propose to solve this

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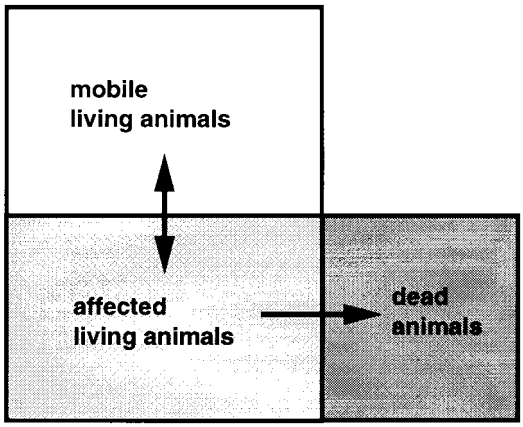

Figure 1. Classical approach to classify arthropod behaviour following insecticide poisoning. Animals are assumed to be either healthy, showing no effects, or to be affected, in which case they can show several gradations of impact on their behaviour, the most severe impact being mortality. Affected animals can show recovery.

problem by adopting a different approach in which mortality and behavioural changes are analysed as independent effects. Such independence of behavioural effects and mortality has been suggested repeatedly for effects of pyrethroids.[9–14] An explanation for this may be offered by the fact that the primary effect of pyrethroids is to affect sensory and motor neurons in a reversible way. This is therefore not in itself associated with mortality. Neurotoxic effects may lead, however, to disruption of other physiological functions, such as circulation, internal chemoreception and neurosecretion, which may be essential for survival. Before discussing the consequences of regarding knockdown as a process from which animals may recover, and which is therefore, in principle, independent from mortality, we will discuss some observations on behaviour and neurosecretion supporting this argument.

1.1 Effects on behaviour

Observations on sensory input and muscular activation have shown that effects are reversible both in relation to toxicant concentration and in relation to temperature-dependent binding of toxicant molecules to neural targets. Reversibility in relation to concentration is, for instance, supported by studies in which house flies were topically treated with different doses of phenothrin and allethrin.[12] The flies in this study showed different gradations of affected behaviour, which was followed by recovery of all animals. Reversibility of effects has also been observed in relation to temperature. Naharashi[13] could evoke the block of axonal conduction by lowering the temperature of a bathing fluid containing allethrin and reverse the effect by raising the temperature again. Here, a physical principle explaining the reversibility of the knockdown-recovery process is offered by the negative temperature dependence of the amount of toxicant molecules bound to target sites at the sodium channels of nerve cells. In line with these observations, we will regard knockdown hereafter as the high-effect phase of a reversible inhibition of locomotor capacity and refer to the linked phases as the knockdown-recovery process, in short the kr-process. The dynamics of reversibility may be affected when the neurons have become physiologically damaged and require long repair periods as well as when topical application results in a high and very local initial concentration which rapidly declines. We will return in the discussion section to the contribution of such dynamics to the kr-process.

1.2 Effects on neurosecretion

Although the impact on sensory and motor neurons may directly affect locomotion, there are several studies which deny a direct link with mortality. For example, the paralysis of circulatory functions and overall nervous activity is not enough to kill insects rapidly as was demonstrated by Gerolt[15] who showed that house flies can fully recover from prolonged periods of neural paralysis due to anoxia. A lack of correlation between knockdown and mortality was also shown by Ford *et al*[11] who studied the effects of a range of different pyrethroids on mustard beetles. Furthermore, the work of Scott and Matsumura[16] revealed two distinct types of action for different pyrethroids showing that 'Type I' action was associated mainly with knockdown, and 'Type II' action with toxicity.

It seems therefore that mortality and toxic effects on motor neurons are poorly linked. A possible explanation is offered by the effect of toxicants on those parts of the nervous system which are involved in neurosecretion. Experimental support for effects on neurosecretion has come from the work of Casida and Maddell,[17] Singh and Orchard[2] and others. An example of a neurosecretory disturbance, for which there are strong indications that it plays a major role in the actual mortality of arthropods following pyrethroid poisoning under different hygrothermal conditions, is the production of diuretic hormone and subsequent water loss.[18–24] A causal relationship between pyrethroid-induced diuretic hormone production and water excretion has been demonstrated by Greenwood *et al*[25] showing that desert locusts from which the *corpora cardiaca* which produce the diuretic hormone had been eliminated by means of radiocautery failed to show the induction of water loss that was observed in intact individuals. But many other neurosecretory disturbances have been shown in poisoned animals.[26,27] With respect to the independence of effects, the above indicates that the involvement of the locomotor and neurosecretory parts of the nervous system allows for considerable differences in response. This could explain the reversibility of the direct toxicant effects on all neurons, this reversibility being independent of the much less reversible or even irreversible effects on the animal's neuroendocrine physiology.

1.3 Consequences of independent effects for knockdown

The above differences in effects on behaviour and physiology suggest strongly that all nerves follow the reversible kr-process, mortality resulting mainly from secondary toxico-dynamics after disturbance of neurosecretory cells. Such a separation of effects allows a description of processes as shown in Fig 2A. Here, the reversible kr-process takes place in living and dead animals alike, whilst mortality occurs selectively in immobile animals. A practical example of selective mortality in immobile animals is desiccation due to pesticide-induced water loss, which has no lethal effects as long as animals can walk and drink. Another example of selective mortality in immobile animals, which, however, involves external causes, is predation on such animals. In addition to the situation where recovery and mortality are in principle independent, whilst mortality still depends on knockdown, one could also imagine a situation in which the lethal activity of the toxicant and its effects on behaviour are fully independent, as is shown in Fig 2B. Here every living animal, whether mobile or immobile, has the same probability of dying. In practice, the two situations differ only in their dynamics, because at a given internal concentration, the latter case shows more mortality, because this acts simultaneously on mobile and immobile animals.

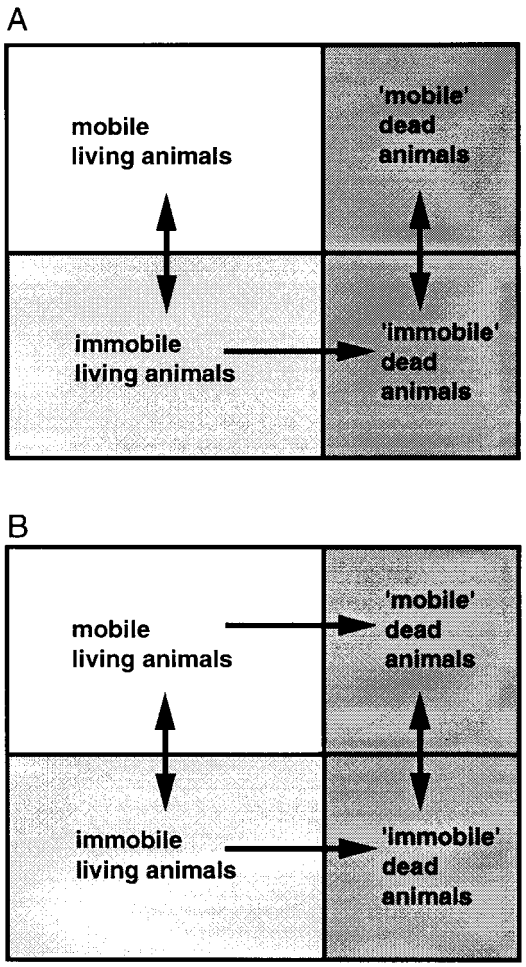

Figure 2. Graphical representation of the partitioning of animals over behaviour and mortality as two independent syndromes. The surface of each part of the graphs represents the percentage of animals of the test population in that class. Figure 2A represents the situation in which effects on behaviour are fully reversible and therefore independent of mortality, whilst mortality depends on affected behaviour. Figure 2B represents the situation in which effects on behaviour are fully reversible and mortality does not depend on behaviour, as it may occur in normal and affected animals alike. Due to independence of the effects, dead animals in both cases are assumed to show changes between a dead–mobile and a dead–immobile state.

If behavioural effects and mortality are independent, this implies that the class of dead animals used in effect studies has to be split up into separate behavioural classes, and that the proportion of the dead animals showing specific behaviour has to be added to the living animals of that class. Of course, there is no direct way to distinguish a dead 'mobile' from a dead 'immobile' animal. The assumption of independent effects, however, offers a way to calculate, for instance, the fraction of animals that is immobile and dead, because this fraction has to be the same as in the living animals. Accordingly, the number of dead immobile animals is given by the following equation:

$$D(I) = D \cdot \frac{S(I)}{S} \qquad (1)$$

in which: $D(I)$ is the number of dead, immobile animals, D the number of dead animals, $S(I)$ the number of surviving, immobile animals and S the total number of surviving animals. The total number of immobile animals in the whole experiment is now given by the sum of $S(I)$ and $D(I)$. Due to the latter addition, the assumption of independent effects may have a large impact on the total number of animals that is considered immobile, especially when high mortality is observed. When more effect classes are used than in the above example, the correction of behavioural observations acts according to the same principle, the exact analysis of such data depending on the definitions of the effect classes. With respect to a graphical representation of the data, we consider it most informative for dynamic analysis of effects if the borderline of each behavioural class is regarded as the limit indicating all animals showing equal or more severe effects. As a consequence, light effects are more inclusive than heavy effects. In this way, a time series of observations will depict the graded response to a pesticide as if we observe 'isoclines' of effect, and the relative distance between the isoclines gives detailed information about the temporal partitioning of animals over the different classes.

2 APPLICATION OF A DEPENDENT AND INDEPENDENT ANALYSIS TO EXPERIMENTAL DATA

To study the effect of analysing knockdown and mortality by a dependent approach, we analysed two different data sets containing detailed observations of effects in time. In both cases effects were split up

into mobile, uncoordinated moving, immobile and dead. The first data set was based on an experiment using adult female linyphiid spiders of the species *Oedothorax apicatus* (Blackwall). These were exposed topically to 2.5 ng per spider of the pyrethroid deltamethrin (Decis-flow, Hoechst Holland BV, Amsterdam) at 10°C. The average weight of the spiders was *c.* 3 mg. Following treatment, they were exposed to different air humidity regimes varying from 100 *via* 85, 70, 55 and 33 to 12.5% RH. Spiders were acclimatised for at least 24 h before the onset of the experiment. Following *ad libitum* food supply, no food was available from 24 h before application and onwards. During the experiments, mobile spiders had the opportunity to drink from small plastic tubes filled water and closed at one end with plaster of Paris.

The impact of deltamethrin on the spiders under the different air humidity regimes is shown in Fig. 3. In the figure a comparison is made between the treatment of data assuming dependence (Figs 3A and B) and independence (Figs 3C and D) of behavioural effects from mortality (Fig 3E). Part A of the figure shows the number of spiders that were alive and showing uncoordinated behaviour or more severe effects. Part B shows the number of living spiders that were classified as immobile. If we now compare part A with part C, the shift from a dependent to an independent viewpoint on effects implies that the number of animals which, in principle, show uncoordinated behaviour is not reduced any more by mortality. Consequently, uncoordinated behaviour is much more severe, and lasts much longer than would be concluded from part A. In principle, the same holds for the number of spiders that are knocked down. However, in addition to a slight increase in effects for all dosages, a strong increase in intensity and duration of knockdown shows up at the lowest air humidities. Because so many animals died, especially at the low air humidities, this long-lasting knockdown effect was completely absent from the conventional analysis. The argument that accidental observational errors due to small numbers of survivors at the lowest air humidity may have caused the complete lack of recovery must be rejected. Long-lasting knockdown is also observed at 33% and to a lesser extent 55% RH, at which air humidities survival was high enough to obtain reasonable estimates of the spiders being knocked down or uncoordinated relative to all living spiders.

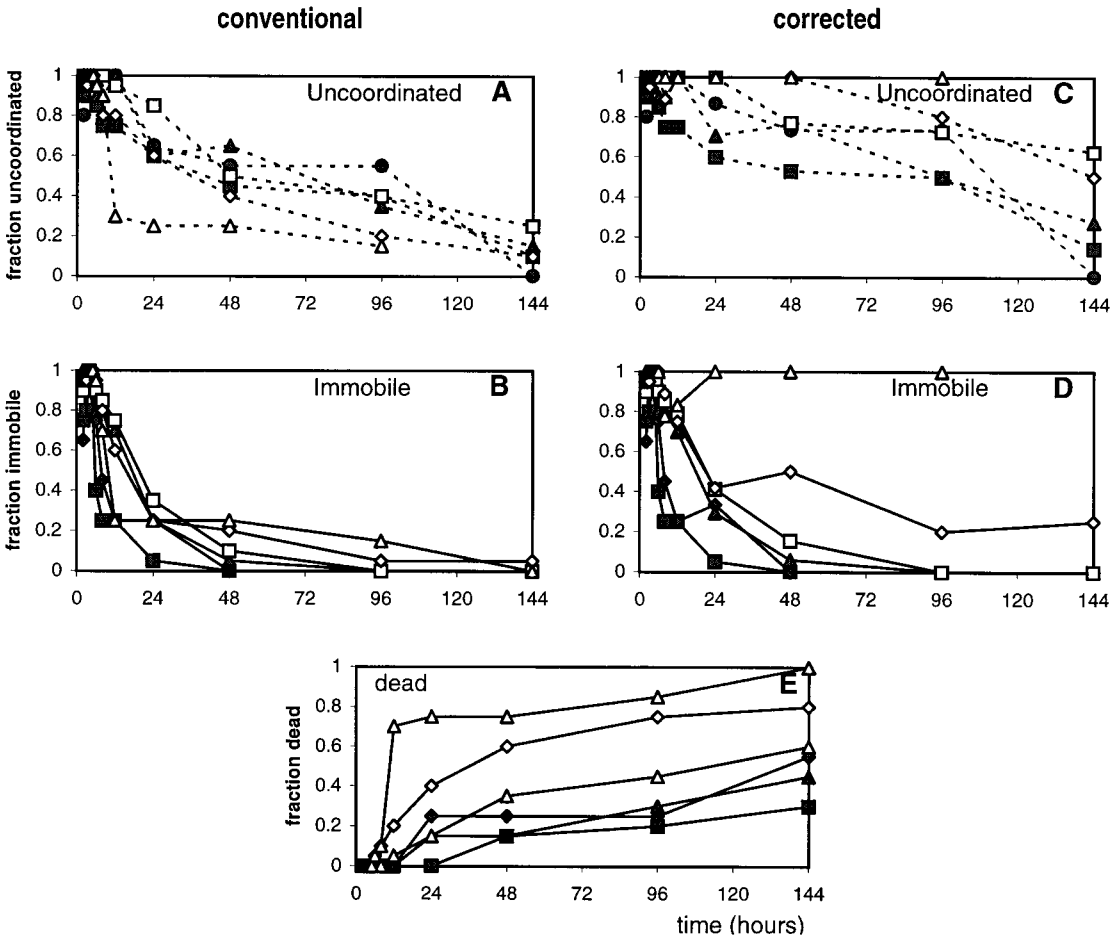

Figure 3. Comparison of the classical effects assessment ('observed') and the presently proposed independent approach ('corrected') for describing effects of deltamethrin poisoning on adult female linyphiid spiders of the species *Oedothorax apicatus*. Experiments conducted at 10°C, 2.5 ng AI per spider. Air humidity of (■) 100, (▲) 85, (◆) 70, (□) 55, (△) 33 and (◇) 12.5% RH. Spiders classified as 'uncoordinated' were able to walk unsteadily when prodded with a fine brush. Spiders classified as 'immobile' could not right themselves. 'Dead' spiders showed no recovery in subsequent observations and became mouldy.

In the second data set, equal numbers of males and non-pregnant females of the beetle *Gastrophysa polygoni* (L.) *c.* 12 mg in weight, which were exposed topically to a mixture of 45 ng of cypermethrin and 0, 310, 1600, 5000 or 10 000 ng prochloraz per individual. Experiments were conducted at 20°C in Petri dishes with moist filter paper on the bottom, at 70% air humidity, long-day conditions (16 : 8 L : D) and unlimited fresh leaf material to feed on.

Although knockdown was observed much earlier, effects were scored for the first time in this study after 24 h. This observational time-lag must be considered the reason for the sharp angle in Figs 4A to 4D at 24 h after treatment. Again a comparison is made in the figure between dependent (Figs 4A and B) and independent (Figs 4C and D) analysis of knockdown and mortality (Fig 4E). The conventional approach shows marked differences between treatments in the numbers of uncoordinated and immobile animals. If more prochloraz is added, fewer animals show affected behaviour, which justifies the conclusion that prochloraz has an antagonistic effect on knockdown. Application of the independent viewpoint by correcting knockdown for mortality leads to a very different conclusion. As is shown in Fig 4D, after correction for mortality the effect of different doses of prochloraz has disappeared. Immobilisation has become approximately the same for all doses of prochloraz. This indicates that prochloraz increases mortality, but does not interfere with effects on behaviour which, caused by cypermethrin in the first place, can be singled out now as being selectivity caused by cypermethrin.

3 DISCUSSION

It is clear from the above examples that conclusions may differ considerably between the dependent and independent viewpoints. Especially in cases of high mortality, the differences between the two methods are large. Yet, as we suggested in the Introduction, there are good reasons to attempt the explanation of effects on behaviour using an independent approach.

The examples in this paper show that the independent viewpoint will, in particular, affect the interpretation of knockdown in studies with high mortality. If no mortality is observed, the two methods give identical results. To investigate further the value of the independent approach in experiments where early mortality occurs, we propose to proceed along two lines. Firstly, it may be informative to re-analyse existing data sets to see in which cases this leads to differences in interpretation of the results. Secondly,

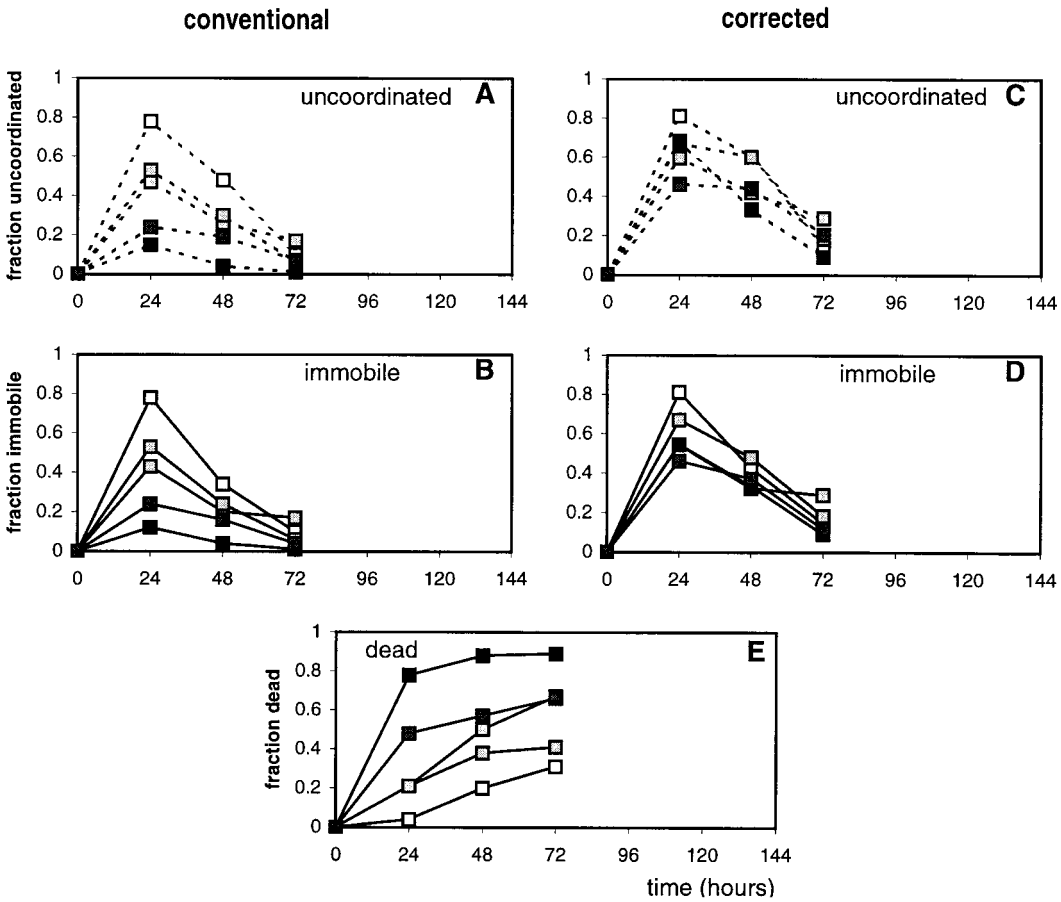

Figure 4. Comparison of the classical effects assessment ('observed') and the presently proposed independent approach ('corrected') for describing effects of cypermethrin poisoning on male and female (non-pregnant) beetles of the species. *Gastrophysa polygoni*. Experiments conducted at 20°C and near 70% RH. Darker shadings represent increasing doses of prochloraz added at the same time varying from 0, to 310, 1600, 5000 and 10 000 ng AI per individual. The classifications 'uncoordinated', 'immobile' and 'dead' are the same as in Fig 3.

we propose to test the value of the independent approach in simulation studies. We consider the independent approach a strong tool for simulations because it can be applied in the same way to situations with and without mortality, and because its assumption of independent effects offers a basis for simulations in which toxico-kinetics independently cause reversible effects on behaviour on the one hand, and dose-related disturbance of neurophysiology as the main cause of mortality on the other. To allow dynamic simulation of dose-related effects of pesticides we advocate the use of 'hazard rates'. Hazard rates describe the probability per period that an animal will shift behavioural state when exposed to a certain toxicant concentration.[28] Examples of such shifts are the transition from alive to dead and the transitions in the reversible process from mobile to immobile, and *vice versa*.[24] It has already been shown that the hazard-rate approach can be applied with good success to aquatic pesticide bioassays.[29]

Due to corrections for mortality in the independent approach, the relationship between toxico-kinetics and effects on behaviour and survival must be considered to reflect the actual events more accurately than when a dependent interpretation of the data is used. In fact, we expect that the use of corrected values will even allow tests of the toxico-kinetics-based hypothesis that the kr-process following topical application in larger animals will show two separate phases. In the initial phase, the nerves just below the local area to which the pesticide was applied respond fiercely, because they are exposed to a high, local concentration. Behavioural responses in this phase are typically observed within minutes after application of the pesticide.[7–9,12,30–32] Such observations strongly suggest peripheral action because toxicants are unlikely to be able to reach internal nerve targets in such a short time, as can be concluded from penetration studies.[13,23,33,34] Due to the dominant role of physicochemical processes in penetration, effects on behaviour in this early penetration phase are highly reproducible. For this reason they have been used with good success as a measure for penetration of pesticides into the cuticle, for instance by Kobayashi *et al*[35] and Nishimura *et al*.[12] Next, and depending on the penetration rate of the compound as mediated by physicochemical properties of the chemical and the cuticle, as well as by hemolymph transport, the locally applied pesticide penetrates into the body and the concentration at the treated spot is reduced. Accordingly, acute effects on behaviour caused by local exposure should decrease. Suggestions for a first phase in toxico-kinetics due to local penetration into the arthropod can be found in the work of Ford and Pert[30] and Elliott *et al*[33] who used topical application to treat arthropods with pyrethroids. The penetration of the pesticide was followed over time via rinsing of the exterior of batches of animals with methanol at various intervals after application. In both studies, the concentration in the external rinse showed an initial sharp dip before it increased again to show a long-lasting slow decline. Without excluding the possibility of other explanations, such a pattern is consistent with local penetration and, later, reappearance of the toxicant in the untreated cuticle.

A second toxico-kinetic phase occurs when the pesticide has reached dynamic equilibrium in all body tissues, which, depending on penetration rate and animal size, will take less than an hour to several hours. At this time all peripheral nerves in the large untreated cuticle area will become affected, but experience a much lower concentration than the initial target site. At that stage, the toxicant will furthermore have penetrated all tissues, including the central nervous system, and the animals' behaviour will show major changes due to simultaneous effects on peripheral and central nerves, the latter including neurosecretory functions. Thus, on toxico-kinetic grounds, we may expect a shift from severe, but highly local effects with short duration, to moderate effects which show a decline in close correlation with the overall decrease in available internal toxicant. It is mainly during this second phase that the neurosecretory targets will be affected, and the secretion of neurohormones will upset physiology thereby causing severe health risks. The relationship between the internal toxicant concentration and late, overall effects on behaviour and neurophysiology is supported by the results of Soderlund[6] and Soderlund *et al*[38] on cockroaches. After topical application of different pyrethroids, these animals showed severe uncoordinated movement and leg tremors in direct correlation with the increase in toxicant concentration measured in the nerve chord. After a short time lag, corresponding with the hypothesis of neuroendocrine disruption, this was followed by excessive diuresis and subsequent reduction in hemolymph volume and full paralysis.

In conclusion, it can be stated that the analysis of the results of two different pesticide bioassays in the present study has shown that it may have a marked impact on the interpretation of the results when mortality and effects on behavioural are analysed as independent effects. For pyrethroids it is argued that the independence results from different consequences of reversible toxicant impacts on all nerves, and of irreversible health effects of neuroendocrine disturbances following toxicant impacts on neurosecretory targets. Differences from the conventional approach are most apparent when experiments show high mortality. It is especially in these situations, therefore, that we expect that an independent approach will improve the relationship between behavioural observations and toxico-kinetics and therewith the ability to analyse and understand the effects of the applied pesticides on arthropods. We strongly advocate the use of combined, detailed observations on behaviour, physiology and toxico-kinetics as a way to gain a better understanding of

the complex processes following pesticide poisoning of arthropods.

REFERENCES

1 Frantik E and Horváth M, Integration of behavioural and neurophysiological approaches in neurotoxicology. *Toxicol Lett* **64/65**:225–229 (1992).

2 Singh GJP, Orchard I, Is insecticide-induced release of insect neurohormones a secondary effect of hyperactivity of the central nervous system? *Pestic Bioch Physiol* **17**:232–242 (1982).

3 Scott JG and Georghiou GP, Influence of temperature on knockdown, toxicity, and resistance to pyrethroids in the house fly, *Musca domestica*. *Pestic Biochem Physiol* **21**:53–62 (1984).

4 Sawicki RM and Thain EM, Insecticidal activity of pyrethrum extract and its four insecticidal constituents against house flies IV: Knock-down activities of the four constituents. *J Sci Food Agric* **13**:292–297 (1962).

5 Sawicki RM, Insecticidal activity of pyrethrum extract and its four insecticidal constituents against house flies III: Knock-down and recovery of flies treated with pyrethrum extract with and without piperonyl butoxide. *J Sci Food Agric* **13**:283–292 (1962).

6 Soderlund DM, Pharmacokinetic behaviour of enantiomeric pyrethroid esters in the cockroach, *Periplaneta americana* L. *Pestic Biochem Physiol* **12**:38–48 (1979).

7 Mullié WC and Everts JW, Uptake and elimination of ^{14}C-deltamethrin by *Oedothorax apicatus* (Arachnida; Erigonidae) with respect to bioavailability. *Pestic Biochem Physiol* **39**:27–34 (1991).

8 Toth SJ and Sparks TC, Effect of temperature on toxicity and knockdown activity of *cis*-permithrin, esfenvalerate and lambda-cyhalothrin in the cabbage looper (Lepidoptera: Noctuidae). *J Econ Entomol* **83**:342–346 (1990).

9 Page ABP, Stringer A and Blackith RE, Bioassay system for the pyrethrins I: Water-base sprays against *Aedes aegypti* L. and other insects. *Ann Appl Biol* **36**:225–249 (1949).

10 Ford M, Quantitative structure–activity relationships of pyrethroid insecticides. *Pestic Sci* **10**:39–49 (1979).

11 Ford MG, Greenwood R and Thomas PJ, The kinetics of insecticide action. Part II: the relationship between the pharmacokinetics of substituted benzyl (1*RS*)-*cis*,*trans*-chrysanthemates and their relative toxicities to mustard beetles (*Phaedon chochleariae* Fab.). *Pestic Sci* **12**:265–284 (1981).

12 Nishimura K, Okajima N, Fujita T and Nakajima, M. Quantitative structure–activity studies of substituted benzyl chrysanthemates. 4: Physicochemical properties and the rate of progress of the knockdown symptoms induced in house fly. *Pestic Biochem Physiol* **18**:314–350 (1982).

13 Naharashi T, Mode of action of pyrethroids. *Bull Wld Hlth Org* **44**:337–345 (1971).

14 Miller TA and Salgado VL, The mode of action of pyrethroids on insects, in *The pyrethroid insecticides*, Ed by Leahey JP, Taylor & Francis, London (1985).

15 Gerolt P, Insecticides: Their route to entry, mechanisms of transport and mode of action. *Biol Rev* **58**:233–274 (1983).

16 Scott JG and Matsumura F, Evidence for two types of toxic action of pyrethroids on susceptible and DDT-resistant German Cockroaches. *Pestic Biochem Physiol* **19**:141–150 (1983).

17 Casida JE and Maddell SHP, Diuretic hormone release on poisoning Rodnius with insecticide chemicals. *Pestic Biochem Physiol* **1**:71–83 (1970).

18 Ingram RL, Water loss from insects treated with pyrethrum. *Ann Entomol Soc Am* **48**:481–485 (1955).

19 Hewlett PS and Gostick KG, The loss of weight of pyrethrum-treated flour beetles, *Tribolium castaneum* Herbst., and its application to bioassay. *Ann Appl Biol* **43**:213–236 (1955).

20 Sun YP and Johnson ER, Dynamics of insecticide and drug effects on the mechanism of water conservation of houseflies and their interactions affecting weight-loss, water balance, intoxication, and recovery of insects. *J Econ Entomol* **65**:667–673 (1972).

21 Gerolt P, The mode of action of insecticides: Accelerated water loss and reduced respiration in insecticide-treated *Musca domestica*. *Pestic Sci* **7**:604–620 (1976).

22 Sundukov OV, Schachmatova EI, Parnova RG and Natochin YV, Relationships between the loss of water and ions in insects due to the action of insecticides. *Tag-Ber Akad Landwirtsch-Wiss DDR Berlin* **274**:31–35 (1989).

23 Greenwood R, Ford MG, Peace EA and Salt DW, The kinetics of insecticide action. Part IV: The in-vivo distribution of pyrethroid insecticides during insect poisoning. *Pestic Sci* **30**:97–121 (1990).

24 Jagers op Akkerhuis GAJM, Rossing WAH, Piet GI and Everts JW, Water depletion, an important cause of mortality in females of the spider *Oedothorax apicatus* after treatment with deltamethrin; a simulation study. *Pestic Biochem Physiol* **58**:63–76 (1997).

25 Greenwood R, Marshman SA and Ford MG, Neurotoxic effect of permethrin on water loss in *Schistocerca gregaria*, in *Neuropharmacology and pesticide action*, *Neurotox '85*, pp 459–550, Society of Chemical Industry, London (1986).

26 Shankland DL and Kearns CW, Characteristics of blood toxin in DDT-poisoned cockroaches. *Ann Entomol Soc Amer* **52**:368–394 (1959).

27 Maddrell HP, The insect neuroendocrine system as a target for insecticides, in *Insect neurobiology and pesticide action*, *Neurotox '79*, p 329, Society of Chemical Industry, London (1980).

28 Bedaux JJM and Kooijman SALM, Statistical analysis of bioassays, based on hazard modeling. *Environ Ecotoxicol Statistics* **1**:303–314 (1994).

29 Kooijman SALM and Bedaux JJM, *The analysis of aquatic toxicity data*, VU University Press, Amsterdam (1996).

30 Ford MG and Pert DR, Time–dose–response relationships of pyrethroid insecticides with special reference to knockdown. *Pestic Sci* **5**:635–641 (1974).

31 Kobayashi T, Nishimura K, Ohoka M and Fujita T, Quantitative structure–activity studies of pyrethroids 19. The rate of development of the knockdown symptom in house-flies (II). *Pestic Biochem Physiol* **35**:275–283 (1989).

32 Schnitzerling HJ, Nolan J and Hughes S, Toxicology and metabolism of some synthetic pyrethroids in larvae of susceptible and resistant strains of the cattle tick *Boophilus microplus* (Can.). *Pestic Sci* **14**:64–72 (1983).

33 Elliott M, Ford MG and Janes NF, Insecticidal activity of the pyrethrins and related compounds. III Penetration of pyrethroid insecticides into mustard beetles (*Phaedon cochleariae*) *Pestic Sci* **1**:220–223 (1970).

34 Zhong H, Hastings FL, Hain FP, Holstein EH and Werner RA, The rate of penetration and residual toxicokinetics of carbaryl on southern pine beetle and spruce beetle (Coleoptera: Scolytidae). *J Econ Entomol* **88**:543–550 (1995).

35 Kobayashi T, Nishimura K, Ohoka M and Fujita T, Quantitative structure–activity studies of pyrethroids. 19. The rate of development of the knockdown symptom in house fly. *Pestic Biochem Physiol* **35**:275–283 (1989).

36 Soderlund DM, Hessney CW and Helmuth DW, Pharmacokinetics of *cis*- and *trans*-substituted pyrethroids in the American cockroach. *Pestic Biochem Physiol* **20**:161–168 (1983).